Scaphanocephalus spp. (Trematoda: Opisthorchiidae) in intermediate and definitive hosts of the Yucatán Peninsula, Mexico, with a re-description of Scaphanocephalus expansus

M. T. González-García[1,2] 🆔, M. García-Varela[1] 🆔, A. López-Jiménez[1] 🆔,
M. P. Ortega-Olivares[1] 🆔, G. Pérez-Ponce de León[3] 🆔 and L. Andrade-Gómez[3] 🆔

[1]Departamento de Zoología, Instituto de Biología, Universidad Nacional Autónoma de México, Avenida Universidad 3000, Ciudad Universitaria, C.P. 04510, Ciudad de México, México; [2]Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México, Avenida Universidad 3000, Ciudad Universitaria, C. P. 04510, México and [3]Departamento de Sistemas y Procesos Naturales, Escuela Nacional de Estudios Superiores Unidad Mérida, Km 4.5, Carretera Mérida-Tetiz, Ucú, Yucatán, C.P. 97357, México

Summary

Scaphanocephalus is a small trematode genus belonging to the family Opistorchiidae. The genus currently contains only three species associated with marine fish as intermediate hosts and fish-eating birds as definitive hosts. Here, specimens of *Scaphanocephalus* were collected from the Osprey, *Pandion haliaetus*, and the White mullet, *Mugil curema* in the Yucatán Peninsula, Mexico. We report for the first-time DNA sequences of adult specimens of *Scaphanocephalus*, particularly *S. expansus*, as well as a sequence of a different species sampled as metacercaria. Morphological comparisons of *Scaphanocephalus expansus* confirmed the identity of the adult specimens, with minor morphological variations; Scanning electron photomicrographs were included, and the species was re-described. Phylogenetic analysis based on 28S rDNA sequences showed that *Scaphanocephalus* is monophyletic within Opisthorchiidae and consists of three independent lineages. Sequences of adults are identical to those of *S. expansus*. Instead, the sequence of the metacercaria sampled from the mesentery of *Mugil curema* nested with specimens reported as *Scaphanocephalus* sp. from a labrid fish in the Mediterranean Sea, herein named it as *Scaphanocephalus* sp. 2.

Introduction

The opisthorchiid trematode genus *Scaphanocephalus* Jägerskiöld, 1904 comprises only three species which exhibit a complex life cycle involving marine snails as their first intermediate hosts, marine fishes as second intermediate hosts, and fish-eating birds from the order Accipitriformes as definitive hosts (Kohl *et al.* 2019; Sokolov *et al.* 2021). They are characterized by a distinctive T-shaped form of the body resembling a cross-section of a mushroom (Pearson, 2008; Katahira *et al.* 2021).

Scaphanocephalus expansus (Creplin 1842) [type-species] was originally described as *Monostomum expansum* from the intestine of the Osprey, *Pandion haliaetus* (L.) (Pandionidae) in Europe. Subsequently, Jägerskiöld (1904) reassigned those specimens and new material collected from the same host in the Red Sea, to the genus *Scaphanocephalus*. Adult specimens of this species seem to be highly host-specific since they have been reported in the Red Sea, Mediterranean Sea, Gulf of California, Gulf of Mexico, and Canary Archipelago, only parasitizing the Osprey, *P. haliaetus* (Creplin, 1842; Jägerskiöld, 1904; Hoffman *et al.* 1953; Dubois, 1960; Schmidt & Huber, 1985; Kinsella *et al.* 1996; Foronda *et al.* 2009). In contrast, larval stages of *S. expansus* have been observed on fins and scales of 17 species of reef fish allocated in 12 families in the Western Pacific, Caribbean Sea, and Gulf of Mexico (Yamaguti, 1942; Kohl *et al.* 2019; Montoya-Mendoza *et al.* 2021). The metacercariae of *Scaphanocephalus* causes the black-spot syndrome (Elmer *et al.* 2019; Kohl *et al.* 2019; Cohen-Sánchez *et al.* 2023).

The other two congeneric species are *S. australis* Johnston, 1917 reported from the White-bellied Sea Eagle, *Haliaeetus leucogaster* (Gmelin) (Accipitridae) in Australia (WoRMS, 2023), and *S. adamsi* Tubangui, 1933 which was described solely from the metacercarial stage obtained from the fins and under the scales of the Splitlevel hogfish, *Bodianus mesothorax* (Bloch & Schneider) (Labridae) in the Philippines (Yamaguti, 1958). Even though Yamaguti (1942) suggested that *S. adamsi* represented the same species as *S. expansus*, it has been also reported from marine fishes of the families Mullidae and Scaridae in Japan (Shimose *et al.* 2020; Katahira *et al.* 2021). Additionally, the metacercariae of *Scaphanocephalus* sp. (unidentified) has been recorded as parasite of marine fishes belonging to six families, i.e., Tetraodontidae, Serranidae, Hemiramphidae,

Pomacentidae, Siganidae, and Labridae, in localities of the Northeast Pacific Ocean, Gulf of Mexico, Gulf of California, Arabian Gulf and Mediterranean Sea (Pérez-Urbiola *et al.* 1994; Bullard & Overstreet, 2008; Al-Salem *et al.* 2021; Cohen-Sánchez *et al.* 2023).

Until now, 28S rDNA sequences have been obtained only for the metacercarial stages of *Scaphanocephalus* spp. We collected metacercariae of *Scaphanocephalus* from the body cavity of one out of 67 specimens of White mullet, *Mugil curema* (Mugilidae) sampled in three coastal lagoons of Yucatán. Additionally, adult specimens of *Scaphanocephalus* spp. were collected from the intestine of one specimen of the Osprey, *Pandion haliaetus* from Champotón, Campeche, also in the Yucatán Peninsula. Adults were morphologically identified as *Scaphanocephalus expansus.* Thus, the main objective of this study was two-fold: to corroborate molecularly the identification of the adults, and to establish a molecular link between the metacercariae and adults from the same geographical region.

Materials and methods

Specimen collection

Adult specimens of *Scaphanocephalus* sp. were recovered from the intestine of one specimen of *Pandion haliaetus* collected in Champotón, Campeche. Additionally, we collected 67 specimens of the White mullet, *Mugil curema* (Valenciennes) in four coastal lagoons of northern Yucatán, namely La Carbonera, Dzilam de Bravo, Ría Lagartos, and Celestún (Fig. 1). Only one fish was found to be infected in the mesentery with an individual of *Scaphanocephalus* sp. from Celestún. All *Scaphanocephalus* specimens were fixed in nearly boiling distilled water and preserved in 100% ethanol for DNA analyses. Some adult specimens were fixed in hot 4% formalin solution for morphological studies and Scanning Electron Microscopy (SEM) studies.

Morphological analyses

Specimens of *Scaphanocephalus* were stained with Mayer's paracarmine, dehydrated in a graded ethanol series, cleared with methyl salicylate, and mounted on permanent slides with Canada balsam. Mounted specimens were examined and measured using a Leica DM 1000 LED compound microscope (Leica Microsystems CMS GmbH, Wetzlar, Germany). Measures are reported in micrometers (μm). Illustrations of internal morphological features were generated using a drawing tube attached to a Leica MC120HD. Drawings were processed in Adobe Illustrator 27.9 (Adobe, Inc). Voucher specimens, including *hologenophores* (Pleijel *et al.* 2008) (Fig. 2), were deposited in the Colección Nacional de Helmintos (CNHE), Instituto de Biología, Universidad Nacional Autónoma de México (UNAM), Mexico City. Additionally, three adult specimens preserved in 4% formalin, dehydrated in a graded ethanol series, critical point dried, sputter-coated with gold and examined with a Hitachi Stereoscan Model S-2469N scanning electron microscope

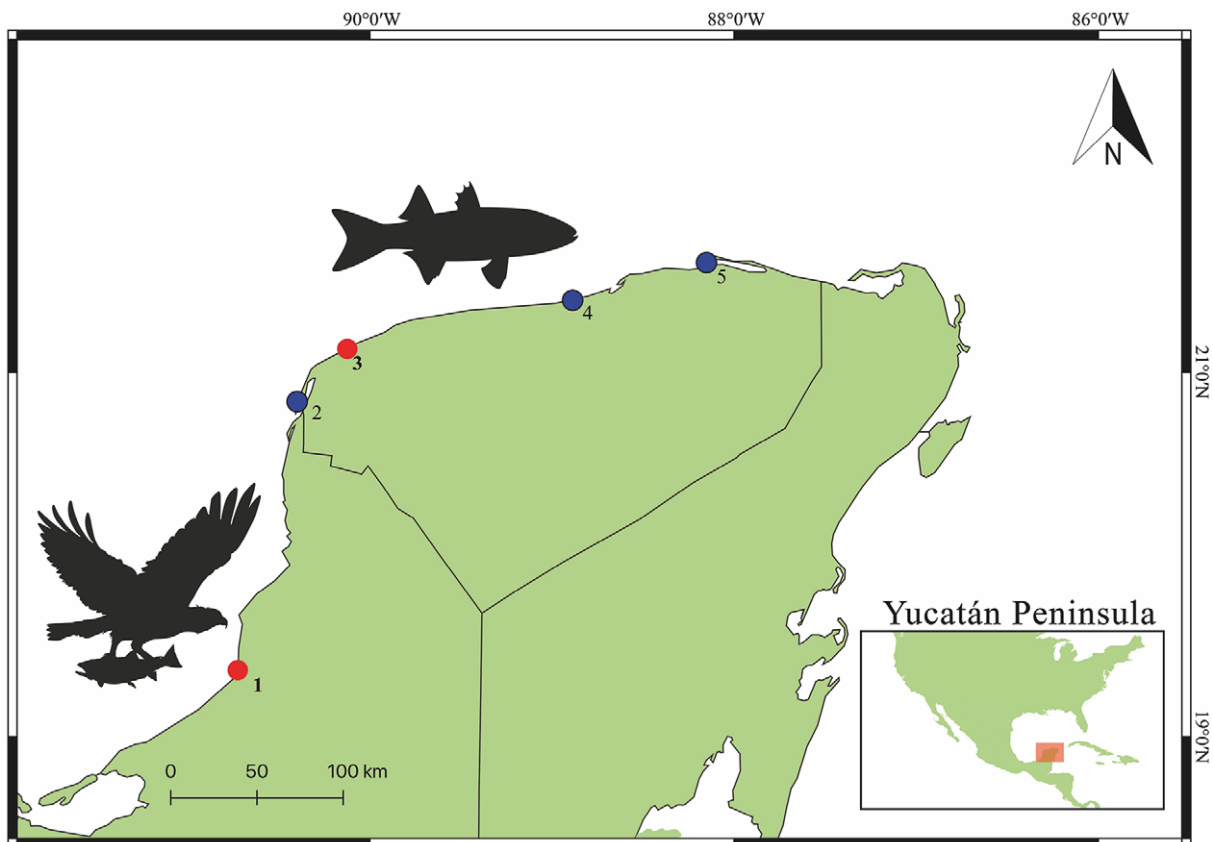**Figure 1.** Sampling collection in Yucatán Peninsula, Mexico. *Pandion haliaetus*, 1. Champotón, Campeche (19º21'40"N, 90º43'5"W). *Mugil curema*, 2. La Carbonera (21º8'1"N, 90º7'55"W) 3. Celestún (20º50'53N, 90º24'22"W), 4. Dzilam (21º23'40"N, 88º53'13"W) 5. Ría Lagartos (21º35'45"N, 88º8'48"W), Yucatán. Localities where *Scaphanocephalus* spp. were recovered are in red. In blue, localities where the parasite was not found.
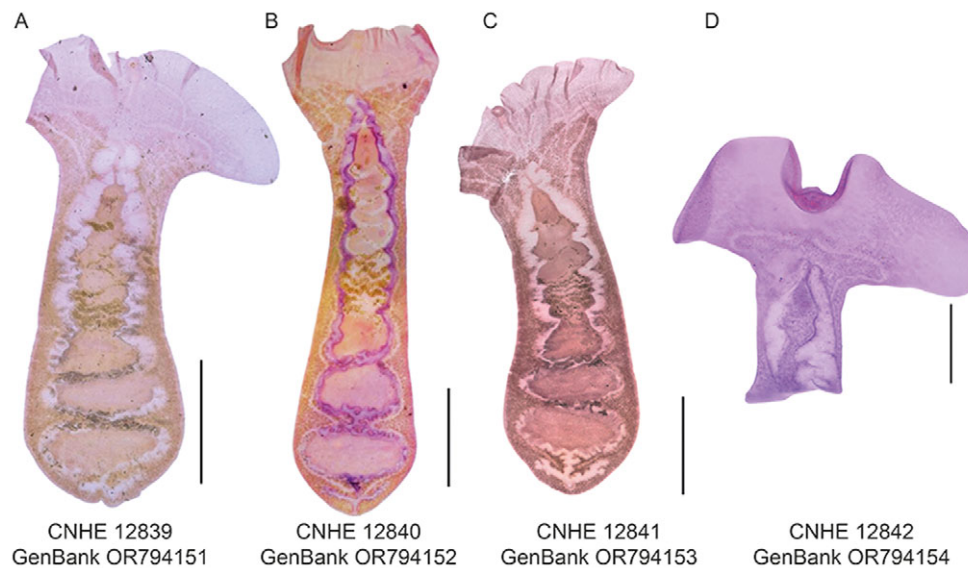
Figure 2. A–C) *Hologenophore* of *Scaphanocephalus expansus* from *Pandion haliaetus* of Champotón, Campeche. D) *Hologenophore* of *Scaphanocephalus* sp. from *Mugil curema* in Celestún, Yucatán. CNHE and GenBank accession numbers are indicated. Scale bars= (A–C) 1 mm, D 300 μm.

at 15 kV at the LaNABIO, Instituto de Biología, Universidad Nacional Autónoma de México (UNAM).

DNA isolation, amplification, and sequencing

Four *hologenophores*, including three adults and one metacercaria, were placed individually in tubes, digested, and DNA extracted following the protocol by González-García *et al.* (2020). Domains D1-D3 from LSU were amplified using forward primer 391, 5-′-AGCGGAGGAAAAGAAACTAA-3′ (Nadler *et al.* 2000), and reverse primer 536, 5′-CAGCTATCCTGAGGGAAAC-3′ (Garcia-Varela & Nadler, 2005). The amplification and sequencing protocols followed those used in Andrade-Gómez *et al.* (2021). Contigs were assembled, and base-calling differences resolved using Codoncode Aligner version 9.0.1 (Codoncode Corporation, Dedham, Massachusetts) and deposited in GenBank.

Alignments and phylogenetic analyses

The newly generated sequences of *Scaphanocephalus* were aligned with other Opisthorchiidae species downloaded from GenBank, along with 14 members of Heterophyidae used as outgroups. Alignment was performed using the software SeaView version 4 (Gouy *et al.* 2010) and adjusted with the Mesquite program (Maddison & Maddison, 2011). A nucleotide substitution model was selected using jModelTest v2.1.7 (Posada, 2008) and applying the Akaike information criterion, with the best nucleotide substitution model for data being GTR + G + I.

Phylogenetic analyses were conducted using Bayesian inference (BI) and maximum likelihood (ML) methods through the online interface Cyberinfrastructure for Phylogenetic Research (CIPRES) Science Gateway v3.3 (Miller *et al.* 2010). The BI analysis was inferred with MrBayes v.3.2.7 (Ronquist *et al.* 2012), with two simultaneous runs of the Markov Chain Monte Carlo (MCMC) for 10 million generations, sampled every 1000 generations, using a heating parameter value of 0.2 and the first 25% of the sampled trees were discarded. The ML analysis was carried out with RAxML v.7.0.4 (Silvestro & Michalak, 2011), and 1000 bootstrap replicates

were run to assess nodal support. Phylogenetic trees were drawn in FigTree v.1.3.1 (Rambaut, 2012). Genetic divergence among taxa was estimated using uncorrected '*p*' distances with MEGA version 6 (Tamura *et al.* 2013).

RESULTS

Class Trematoda Rudolphi, 1808
Order Plagiochiida La Rue, 1957
Family Opisthorchiidae, Looss 1899
Subfamily Cryptocotylinae Lühe, 1909
Genus *Scaphanocephalus* Jägerskiöld, 1904
***Scaphanocephalus expansus* (Creplin, 1842)**

Records: Adult specimens, 1. Creplin (1842); 2. Jägerskiöld (1904); 3. Gohar (1935); 4. Smogorzhevskaya (1956); 5. Dubois (1960); 6. Lee (1966); 7. Schmidt & Huber (1985); 8. Kinsella *et al.* (1996); 9. Hoffman (1999); 10. Foronda *et al.* (2009); 11. This study.

Metacercariae specimens, 12. Yamaguti (1942); 13. Hutton (1964); 14. Skinner (1978); 15. Overstreet & Hawkins (2017); 16. Kohl *et al.* (2019); 17. Elmer *et al.* (2019); 18. Montoya-Mendoza *et al.* (2021).

Type and only definitive host: (1–11) *Pandion haliaetus* (L.) (Pandionidae)

Intermediate hosts: Acanthuridae: (16, 17) *Acanthurus tractus* Poey; (16), *A. chirurgus* Bloch; (16) *A. coeruleus* Bloch & Schneider; Carangidae: (16) *Caranx ruber* Bloch; Gerreidae: (14) *Eucinostomus argenteus* Baird & Girard; Labridae: (18) *Halichoeres radiatus* (L.); Lutjanidae: (15) *Lutjanus griseus* (L.); Monacanthidae: (16) *Cantherhines pullus* Ranzani; Mullidae: (16) *Mulloidichthys martinicus* Cuvier; (12) *Parupeneus multifasciatus* Quoy & Gaimard; Pristidae: (13) *Pristis pectinata* Latham; Scaridae: (16) *Sparisoma aurofrenatum* Valenciennes, (16) *S. chrysopterum* Bloch & Schneider; Sciaenidae: (14) *Micropogonias undulatus* (L.); Serranidae: (16) *Cephalopholis cruentata* Lacepède; (16) *Paranthias furcifer* Valenciennes; Sparidae: (14) *Archosargus rhomboidalis* (L.).

Type locality: (1, 4) 'Europe' (unspecified locality)

Table 1. Comparative morphometric data for *Scaphanocephalus expansus*

Reference	Jägerskiöld, 1904	Dubois, 1960	Foronda *et al.* 2009	Present study
Geographic region	Red Sea	Mediterranean Sea	Canary Archipelago	Gulf of Mexico
Host	Pandion haliaetus	*P. haliaetus*	*P. haliaetus*	*P. haliaetus*
Stage	Adult	Adult	Adult	Adult
Body length	5000	4650–4760	2812.2–3709.2	2256–5063
Anterior wing width	3200	2730–3290	1235.4–2145.2	1177–2792
Hindbody width	–	–	799.3–1052.1	643–1375
Oral sucker legth	128	138	94.8–107.2	64–129
Oral sucker width	96	125	83.2–89.2	74–116
Pharynx legth	80	81–95	69.7–82.7	54–88
Pharynx width	60	78–85	46.5–69.7	40–87
Prepharynx length	–	37–42	72.3–96.3	14–43
Oesophagus length	272	180	162.4–267.4	151–309
Ventrogenital legth	–	–	–	214–415
Ventrogenital width	–	–	–	178–279
Anterior testis legth	480	400–690	521.1–664.5	221–465
Anterior testis width	560	1270–1300	301–449.2	442–912
Posterior testis legth	740	580–840	548.5–719.9	276–495
Posterior testis width	860	1150–1270	339.1–517.3	424–951
Ovary length	160	270–380	223.3–372.9	105–263
Ovary width	400	700–870	134.4–207.4	226–454
Egg length	27	22–40	19.7–30.7	22–24
Egg width	16	16–20	14–21.6	18–21

Other localities: Adult specimen records: (2, 3) Red Sea; (5) Mediterranean Sea; (6) Malaysia; (7) Gulf of California; (8, 11) Gulf of Mexico; (9) USA; and (10) Canary Archipelago. Metacercariae specimen records: (12) Japan; (13, 14, 15, 18) Gulf of Mexico; and (16, 17) Caribbean Sea.

Site of infection: Adults in intestine, Metacercaria on fins and underneath scales

Intensity: Adults specimens: (7) 35; (8) 361; (9) 30; (10) 43; (11) 123.

Specimens deposited: 16 vouchers (CNHE12838); 3 *hologenophores* (CNHE12839–12841)

Representative DNA sequences: OR794151–OR794153 (28S)

Redescription based on 19 specimens, including three *hologenophores* plus three individuals analyzed for SEM (Fig. 2A–C & 3). Measurements are provided in Table 1.

Body elongated and flared anteriorly (Fig. 3A, 3B). Tegument completely armed with two types of spines, pectinate on large wing-like anterior expansions, and arrow-like on posterior body end (Fig. 3D, 3E). Anterior of the body exhibiting prominent wing-like anterior expansions; anterior border crenulated, striated (3B, 3C). Oral sucker subterminal, bearing dome-like papillae (Fig. 3C). Prepharynx inconspicuous; pharynx oval, oesophagus relatively large. Caeca narrow extending to posterior body end reaching the inter-testicular level. Ventrogenital complex located in first third of body. Ventral sucker greatly reduced embedded in body parenchyma, opening into the ventrogenital complex.

Testes two, deeply lobated, in tandem, in posterior third of body. Posterior testis larger. Seminal vesicle tubular, winding, dorsally to uterus. Ovary deeply lobated, post-ecuatorial, pretesticular. Mehli´s gland sinistral, at ovary level. Seminal receptacle postovarian, pretesticular. Laurer's canal between ovary and Mehli's gland. Vitelline glands in the lateral fields, from caecal bifurcation to posterior body end; confluent in post-testicular area. Vitelloducts at ovary level. Uterus pretesticular, deeply coiled, between ventrogenital complex region and ovary, opening to genital pore in ventrogenital complex. Eggs small, oval to round shaped. Excretory vesicle Y-shaped, extending sinuously to reach anterior of body.

Remarks

Our specimens were identified as *Scaphanocephalus expansus* by having features consistent with the diagnosis of the original description and recent re-descriptions (Creplin, 1842; Jägerskiöld, 1904; Dubois, 1960; Pearson, 2008; Foronda *et al.* 2009). For instance, our specimens possess an anterior body with wing-like expansions, testes deeply lobated located in tandem in the posterior third of body, ovary lobated, pretesticular, and tegument covered with spines. In addition, metrical data of our specimens are like those reported in previous studies (Table 1). We noticed, however, some slight morphological differences, such as the variation in body length; the newly sampled specimens are, on average, smaller than
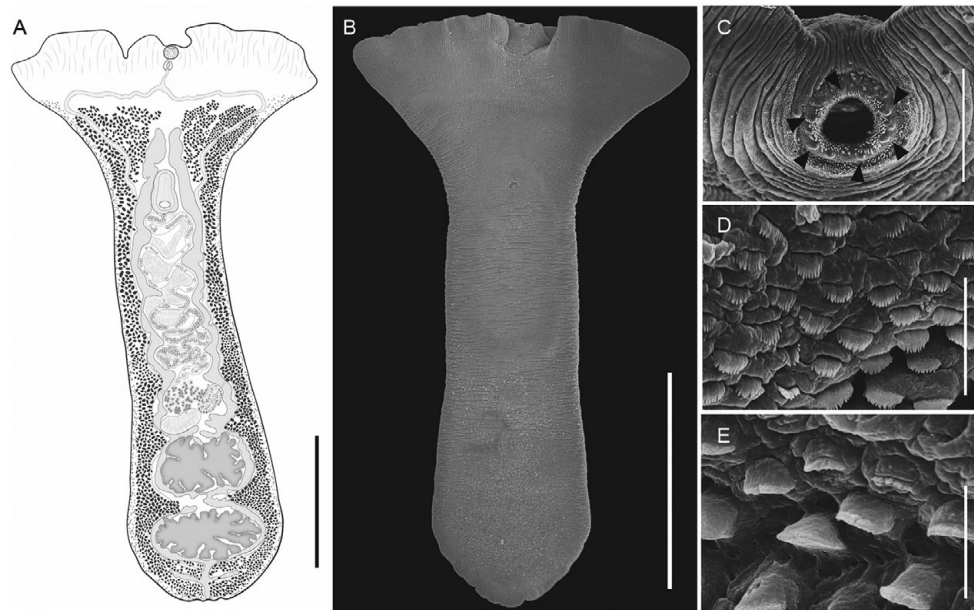
Figure 3. A) *Scaphanocephalus expansus* from *Pandion haliaetus.* Schematic whole worm, ventral view. Scanning electron photomicrographs, B) whole worm; C) Oral sucker; D) Tegumental spines at level of wing-like expansions; E) Spines at the level of the third part of the body. Scale bars = (A, B) 1 mm; (C), 50 μm; (D, E) 5 μm.

those reported in previous studies (2256−5063 *vs* 2812−5000). The ovary and testes are also smaller (Ovary: 105−263 *vs* 160−380; anterior testis: 221−465 *vs* 400−690; posterior testis: 276−495 *vs* 548−840) (Table 1). In addition, SEM images allowed to show that *S. expansus* presented two types of spines, i.e., pectinate and arrow-like (Figs. 3D, 3E). Dome-like papillae surrounding the aperture of the oral sucker are described for the first time (Fig. 3C).

We re-examined the specimens deposited of the metacercariae of *Scaphanocephalus expansus* by Montoya-Mendoza *et al.* (2021) in the Colección Nacional de Helmintos, México City (CNHE No. 11508) reported encysted on the fins of the labrid *Halichoeres radiatus* in the Veracruz coral reef, Mexico; and noticed that they are morphologically very similar to our adult specimens, both with multilobulated testes. However, DNA sequences are required of these larvae stages to corroborate the status and complete the life cycle of the species.

Phylogenetic analyses

The LSU dataset comprised 36 sequences with 1,247 characters. The alignment was trimmed to the shortest and included the four newly generated sequences of *Scaphanocephalus,* and six sequences previously identified either as *Scaphanocephalus* sp., or *S. expansus*. In addition, the alignment included 11 sequences of members of Opisthorchiidae, plus 14 sequences of Heterophyidae. Phylogenetic analyses conducted through BI and ML methods recovered similar topologies (Fig. 4). Analyses showed Opisthorchiidae as monophyletic with strong nodal support (1/100); Cryptocotylinae was also recovered as monophyletic, including *Cryptocotyle* spp. and *Scaphanocephalus* spp. The genus *Scaphanocephalus* was divided in three highly supported clades (1/100). The first clade herein referred as *Scaphanocephalus* sp. 1 (MN160569 and MT461356) included two sequences from a siganid and acanthurid from the Arabian Gulf and the Caribbean Sea, respectively. This clade was resolved as the sister group of the other two clades of *Scaphanocephalus*. The second clade was formed by three of the newly generated sequences of *S. expansus*

(from the definitive host, *P. haliaetus*), plus one sequence previously identified as *S. expansus* (MK680936) sampled from a scarid fish, and one sequence identified as *Scaphanocephalus* sp. (MN160570) from an acanthurid fish, both from the Caribbean Sea. Finally, the third clade was yielded as the sister group of *S. expansus* and is formed by four sequences, the specimen from *Mugil curema* (Fig. 2D; CNHE 12842), and those previously identified as *Scaphanocephalus* sp. from a labrid in the Mediterranean Sea, herein referred as *Scaphanocephalus* sp. 2.

The genetic divergence of 28S estimated among the three clades of *Scaphanocephalus* ranged from 1.06%−1.73%., whereas the divergence between *Scaphanocephalus* sp. 1 (MN160569 + MT461356) and *S. expansus* + *Scaphanocephalus* sp. 2 was 1.39−1.73%. The species pair *S. expansus* and *Scaphanocephalus* sp. 2 varied 1.06−1.12%. The genetic divergence within each clade of *Scaphanocephalus* was null.

Discussion

The genus *Scaphanocephalus* was originally considered a member of Heterophyidae (Pearson, 2008; Dennis *et al.* 2019). However, Sokolov *et al.* (2021) recently transferred *Scaphanocephalus* to the subfamily Cryptocotylinae within Opisthorchiidae using molecular and morphological data. Our LSU phylogenetic analyses unequivocally corroborated that *Scaphanocephalus* is monophyletic and belongs to Opisthorchiidae.

Dennis *et al.* (2019) performed the first molecular phylogenetic analysis including two lineages of *Scaphanocephalus*, recognizing to separate species as *Scaphanocephalus* sp. 1 and *Scaphanocephalus* sp. 2. The latter was nested with *S. expansus* reported by Kohl *et al.* (2019). Al-Salem *et al.* (2021) and Cohen-Sánchez *et al.* (2023) obtained sequences of *Scaphanocephalus* although without a molecular phylogenetic analysis. Unexpectedly, our phylogenetic analysis showed that the sequence of *Scaphanocephalus* from the Arabian Gulf (MT461356) reported by Al-Salem *et al.* (2021)
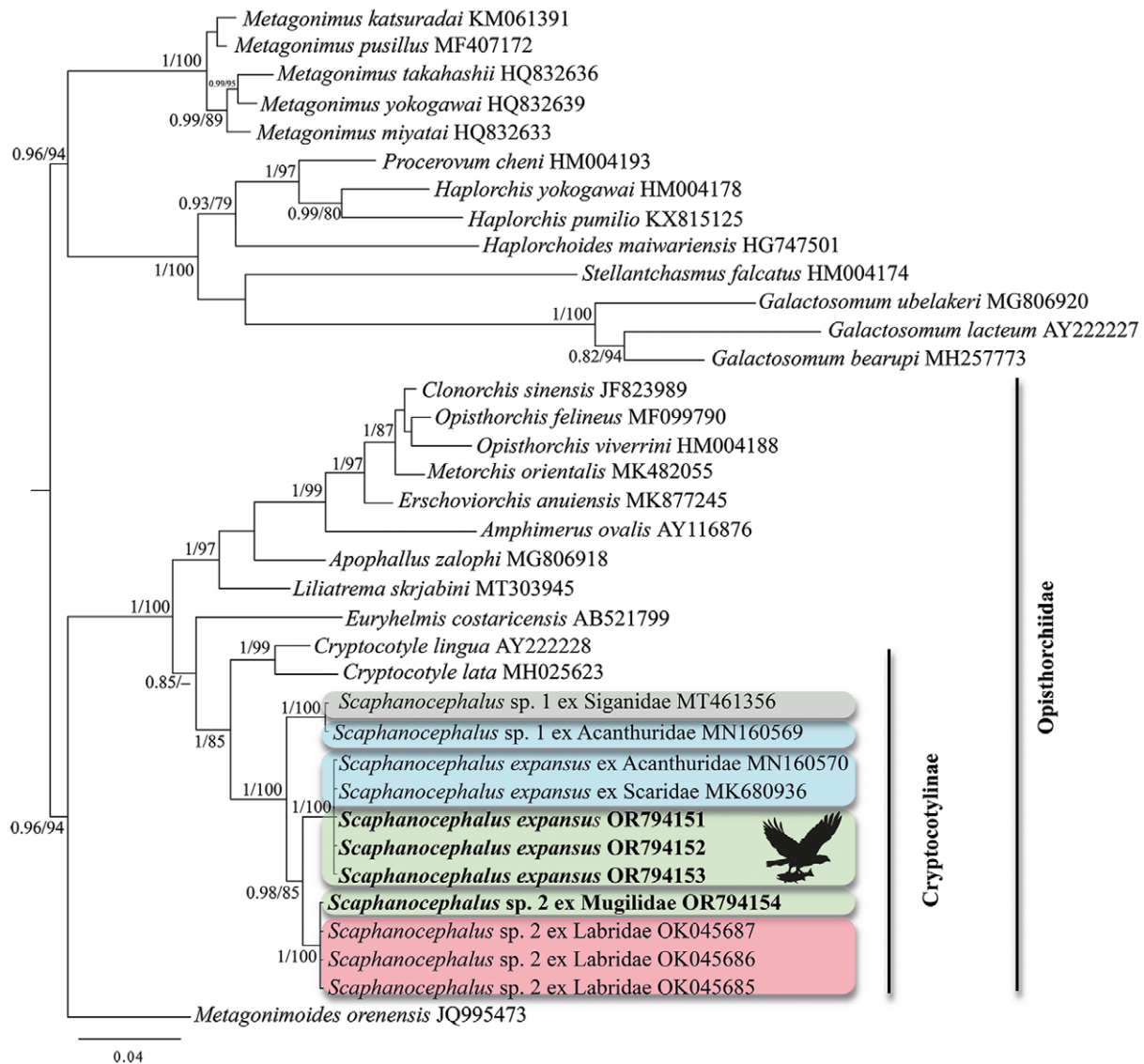
Figure 4. Consensus Bayesian inference (BI) tree and Maximum likelihood (ML) tree inferred from the large subunit from nuclear ribosomal DNA. Numbers on internal nodes show posterior probabilities (BI) and ML bootstrap clade frequencies. Sequences generated in this study in bold. Grey: specimens from Arabian Gulf. Blue: specimens from the Caribbean Sea. Green: specimens from Yucatán Peninsula, Mexico. Red: specimens from the Mediterranean Sea. Scale bar shows the number substitutions per site.

nested with *Scaphanocephalus* sp. 1 from the Caribbean Sea reported by Dennis *et al.* (2019).

Moreover, the sequence reported as *Scaphanocephalus* sp. 2 (MN160570) by Dennis *et al.* (2019) from the acanthurid *A. chirurgus* in the Caribbean Sea nested as sister taxon of *S. expansus* from the scarid *S. chrysopterum* in the same geographical region as reported by Kohl *et al.* (2019). Our sequences from adult specimens of *S. expansus* from the definitive host were also nested within this clade. Based on this evidence, we consider that *Scaphanocephalus* sp. 2 of Dennis *et al.* (2019) as *S. expansus*. Furthermore, Cohen-Sánchez *et al.* (2023) recently reported sequences of *Scaphanocephalus* sp. from the labrid *X. novacula*, in the Mediterranean Sea. These authors made no further taxonomic consideration because their study was focused on determining the effect of the black spot disease produced by *Scaphanocephalus* on the oxidative stress of the host. We included these sequences in our analyses and found that they nested with the newly sequenced individual from *M. curema* from Yucatán,

with null genetic variation, suggesting this lineage is widely distributed across the Atlantic Ocean parasitizing marine fishes. This clade is herein considered as *Scaphanocephalus* sp. 2 (Fig. 4). In addition, *M. curema* represent a new species of intermediate hosts for *Scaphanocephalus*. Interestingly, only one specimen of *Scaphanocephalus* was recovered from the mesentery of 1 out of 67 individuals of *M. curema* studied for parasites in four localities of northern Yucatán. The infection site (mesentery) and the low prevalence of infection suggest that the presence of the parasite in *M. curema* may represent an accidental infection. The metacercariae of *Scaphanocephalus* have been reported as ectoparasites encysted on the fins and under the scales of their hosts (Dennis *et al.* 2019; Elmer *et al.* 2019; Kohl *et al.* 2019; Shimose *et al.* 2020; Katahira *et al.* 2021; Montoya-Mendoza *et al.* 2021; Cohen-Sánchez *et al.* 2023).

The LSU genetic divergence observed among the three clades of *Scaphanocephalus* was relatively low (1.06 to 1.73%). However, these genetic divergence values are like those reported for other

members of the Cryptocotylinae. For instance, Tatonova & Besprozvannykh (2019) reported a genetic divergence of 2% between *Cryptocotyle lingua* (Creplin, 1825) and *C. lata* Tatonova and Besprozvannykh, 2019. Interestingly, in our study we found no genetic divergence within each lineage of *Scaphanocephalus*. These data suggest that definitive and intermediate hosts are playing an important role for the distribution of each lineage of *Scaphanocephalus*.

Up to the present, only three species have been included in the genus *Scaphanocephalus*, namely *S. expansus, S. australis* and *S. adamsi*. The first two were described from their definitive hosts, *S. expansus* from *P. haliaetus*, and *S. australis* from *H. leucogaster*. In contrast, the description of *S. adamsi* was based only on the metacercarial stage from the labrid *B. mesothorax* (Tubangui 1933). In the present study, two unidentified *Scaphanocephalus* (sp.1 and sp. 2) represent larval stages. Whether or not they represent the two species of *Scaphanocephalus* not yet sequenced needs to be tested once molecular studies are conducted on these species. Interestingly, *Scaphanocephalus* sp. 2 contain specimens sampled from *M. curema* in Mexico and, more importantly, from the labrid *X. novacula* from Spain (Cohen-Sánchez *et al.* 2023). The latter represents the same host-type (Labridae) of *S. adamsi*, although the species was described from *B. mesothorax* in the Philippines. Two pieces of information are missing to test the hypothesis that *Scaphanocephalus* sp. 2 represents in fact *S. adamsi*, sampling and sequencing metacercariae from the type locality and, ideally, sampling adult forms from Accipitriformes. Furthermore, the only published record of *S. australis* was from adults collected from the accipitrid *H. leucogaster* in Australia (Johnston 1917), and their larval forms have not been reported from marine fishes in the area. The White bellied sea eagle, *H. leucogaster* has an extensive geographic distribution, extending from the Indian west coast, China, to all over South-East Asia, including Indonesia, Papua New Guinea, and Australia (Shephard *et al.* 2005). It is imperative to collect new material of *S. australis* to determine whether or not *Scaphanocephalus* sp. 1 or 2 belong in that species, or if they represent a separate species whose adults have not been found in Accipitriformes.

The osprey, *Pandion haliaetus* has a cosmopolitan distribution which probably has a key role in the distribution of *S. expansus* across the world. In the Caribbean two subspecies have been reported, the North American osprey, *P. haliaetus carolinensis*, which is migratory, and the non-migratory osprey, *P. haliaetus ridgwayi* (Wiley *et al.* 2014) This could explain the presence of the two lineages of *Scaphanocephalus* in the Caribbean, *S. expansus* and *Scaphanocephalus* sp. 1. Still, gathering new samples of adults and metacercariae from different host species is necessary to test the hypothesis on the potential link between larval forms and adults described from accipitriformes, and to understand the evolution and biogeographic history, as well as the interrelationships and host-parasite interactions of this enigmatic group of digeneans.

References

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